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DATE: Monday, November 24, 2003

Set Name side by side	Query	Hit Count	Set Name result set
	DB=USPT,PGPB,EPAB,DWPI,TDBD; THES=ASSIGNEE; PLUR=YES; OP=ADJ		
L3	troponin same (isolat$ or purif$ or chromotograp$) and sulfhydryl and sulfitolyz$	4	L3
L2	troponin same (isolat$ or purif$ or chromotograp$) and sulfhydryl	15	L2
L1	troponin same (isolat$ or purif$ or chromotograp$) same sulfhydryl	5	L1

END OF SEARCH HISTORY

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Search Results - Record(s) 1 through 15 of 15 returned.

1. Document ID: US 20030166062 A1

L2: Entry 1 of 15 File: PGPB Sep 4, 2003

PGPUB-DOCUMENT-NUMBER: 20030166062
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20030166062 A1

TITLE: Methods and compositions for production of recombinant peptides

PUBLICATION-DATE: September 4, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Gonzalez-Villasenor, Lucia Irene	Baltimore	MD	US	

US-CL-CURRENT: 435/69.1; 530/350

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

2. Document ID: US 20030138907 A1

L2: Entry 2 of 15 File: PGPB Jul 24, 2003

PGPUB-DOCUMENT-NUMBER: 20030138907
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20030138907 A1

TITLE: Purification of human troponin I

PUBLICATION-DATE: July 24, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Conn, Gregory	Cary	NC	US	
Reardon, Brian	Seattle	WA	US	
Zeng, Xianfang	Northborough	MA	US	
Zhang, Chenming	Blacksburg	VA	US	

US-CL-CURRENT: 435/69.1; 435/252.33, 435/320.1, 530/350, 536/23.5

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

3. Document ID: US 20030130224 A1

L2: Entry 3 of 15 File: PGPB Jul 10, 2003

PGPUB-DOCUMENT-NUMBER: 20030130224

PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20030130224 A1

TITLE: Expression of zeta negative and zeta positive nucleic acids using a dystrophin gene

PUBLICATION-DATE: July 10, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Monahan, Sean D.	Madison	WI	US	
Wolff, Jon A.	Madison	WI	US	
Slattum, Paul M.	Madison	WI	US	
Hagstrom, James E.	Madison	WI	US	
Budker, Vladimir G.	Madison	WI	US	
Rozema, David B.	Madison	WI	US	

US-CL-CURRENT: 514/44; 602/13

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments KWIC | Draw. Desc | Image

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4. Document ID: US 20030105017 A1

L2: Entry 4 of 15 File: PGPB Jun 5, 2003

PGPUB-DOCUMENT-NUMBER: 20030105017
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20030105017 A1

TITLE: Purification of human Troponin I

PUBLICATION-DATE: June 5, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Conn, Gregory	Cary	NC	US	
Reardon, Brian	Seattle	WA	US	
Zeng, Xianfang	Northborough	MA	US	
Zhang, Chenming	Blacksburg	VA	US	

US-CL-CURRENT: 514/12; 435/69.1, 530/350

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments KWIC | Draw. Desc | Image

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5. Document ID: US 20020064835 A1

L2: Entry 5 of 15 File: PGPB May 30, 2002

PGPUB-DOCUMENT-NUMBER: 20020064835
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020064835 A1

TITLE: Purification of human troponin I

PUBLICATION-DATE: May 30, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Conn, Gregory	Cary	NC	US	
Reardon, Brian	Seattle	WA	US	
Zeng, Xianfang	Northborough	MA	US	
Zhang, Chenming	Blacksburg	VA	US	

US-CL-CURRENT: 435/71.2; 514/2

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw. Desc | Image

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6. Document ID: US 20020055145 A1

L2: Entry 6 of 15 File: PGPB May 9, 2002

PGPUB-DOCUMENT-NUMBER: 20020055145
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020055145 A1

TITLE: Purification of human troponin I

PUBLICATION-DATE: May 9, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Conn, Gregory	Cary	NC	US	
Reardon, Brian	Seattle	WA	US	
Zeng, Xianfang	Northborough	MA	US	
Zhang, Chenming	Blacksburg	VA	US	

US-CL-CURRENT: 435/69.1; 530/417

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw. Desc | Image

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7. Document ID: US 6589936 B1

L2: Entry 7 of 15 File: USPT Jul 8, 2003

US-PAT-NO: 6589936
DOCUMENT-IDENTIFIER: US 6589936 B1

TITLE: Pharmaceutical compositions comprising recombinant troponin subunits

DATE-ISSUED: July 8, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Thorn; Richard M.	North Easton	MA		
Lanser; Marc E.	Dover	MA		
Moses; Marsha A.	Brookline	MA		
Wiederschain; Dmitri G.	Brookline	MA		

US-CL-CURRENT: 514/12; 435/69.1, 435/70.1, 514/2, 530/350

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments KWIC | Draw Desc | Image

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8. Document ID: US 6586401 B1

L2: Entry 8 of 15 File: USPT Jul 1, 2003

US-PAT-NO: 6586401
DOCUMENT-IDENTIFIER: US 6586401 B1

TITLE: Troponin subunit I fragment and homologs thereof

DATE-ISSUED: July 1, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Thorn; Richard M.	North Easton	MA		
Lanser; Marc E.	Dover	MA		
Moses; Marsha A.	Brookline	MA		
Wiederschain; Dmitri G.	Drighton	MA		

US-CL-CURRENT: 514/13; 530/326

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments KWIC | Draw Desc | Image

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9. Document ID: US 6465431 B1

L2: Entry 9 of 15 File: USPT Oct 15, 2002

US-PAT-NO: 6465431
DOCUMENT-IDENTIFIER: US 6465431 B1

TITLE: Pharmaceutical compositions comprising troponin subunits, fragments and homologs thereof and methods of their use to inhibit angiogenesis

DATE-ISSUED: October 15, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Thorn; Richard M.	North Easton	MA		
Lanser; Marc E.	Dover	MA		
Moses; Marsha A.	Brookline	MA		
Wiederschain; Dmitri G.	Brookline	MA		

US-CL-CURRENT: 514/16; 530/328

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments KWIC | Draw Desc | Image

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10. Document ID: US 6403558 B1

L2: Entry 10 of 15 File: USPT Jun 11, 2002

US-PAT-NO: 6403558
DOCUMENT-IDENTIFIER: US 6403558 B1

TITLE: Pharmaceutical compositions comprising troponin subunits, fragments and analogs thereof and methods of their use to inhibit angiogenesis

DATE-ISSUED: June 11, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Moses; Marsha A.	Brookline	MA		
Langer; Robert S.	Newton	MA		
Wiederschain; Dimitri G.	Brookline	MA		
Wu; Inmin	Boston	MA		
Sytkowski; Arthur	Arlington	MA		

US-CL-CURRENT: 514/12; 514/21, 530/324

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

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11. Document ID: US 6025331 A

L2: Entry 11 of 15 File: USPT Feb 15, 2000

US-PAT-NO: 6025331
DOCUMENT-IDENTIFIER: US 6025331 A

TITLE: Pharmaceutical compositions comprising troponin subunits, fragments and analogs thereof and methods of their use to inhibit angiogenesis

DATE-ISSUED: February 15, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Moses; Marsha A.	Brookline	MA		
Langer; Robert S.	Newton	MA		
Wiederschain; Dimitri G.	Brookline	MA		
Wu; Inmin	Boston	MA		
Sytkowski; Arthur	Arlington	MA		

US-CL-CURRENT: 514/12; 514/2

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

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12. Document ID: US 5948771 A

L2: Entry 12 of 15 File: USPT Sep 7, 1999

US-PAT-NO: 5948771
DOCUMENT-IDENTIFIER: US 5948771 A

TITLE: Method for treating heart failure using tetrapyrroles and metallotetrapyrroles

DATE-ISSUED: September 7, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Danziger; Robert S.	New York	NY		

US-CL-CURRENT: 514/185; 540/145

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments KWIC | Draw Desc | Image

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13. Document ID: US 5846738 A

L2: Entry 13 of 15 File: USPT Dec 8, 1998

US-PAT-NO: 5846738
DOCUMENT-IDENTIFIER: US 5846738 A

TITLE: Synthetic standard for immunoassays

DATE-ISSUED: December 8, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Seidel; Christoph	Weilheim			DE
Bialk; Peter	Eberfing			DE
Von der Eltz; Herbert	Weilheim			DE

US-CL-CURRENT: 435/7.1; 435/13, 435/5, 435/69.3, 435/7.9, 435/7.92, 435/961, 435/967, 435/973, 436/517, 436/518, 436/536 , 436/8, 530/324, 530/325, 530/326, 530/327, 530/328, 530/329, 530/350, 530/806

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments KWIC | Draw Desc | Image

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14. Document ID: US 5837680 A

L2: Entry 14 of 15 File: USPT Nov 17, 1998

US-PAT-NO: 5837680
DOCUMENT-IDENTIFIER: US 5837680 A

TITLE: Pharmaceutical compositions comprising troponin subunits, fragments and analogs thereof and methods of their use to inhibit angiogenesis

DATE-ISSUED: November 17, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Moses; Marsha A.	Brookline	MA		
Langer; Robert S.	Newton	MA		
Wiederschain; Dimitri G.	Brookline	MA		
Wu; Inmin	Boston	MA		
Sytkowski; Arthur	Arlington	MA		

US-CL-CURRENT: 514/12; 514/21, 530/324

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments KWIC | Draw Desc | Image

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15. Document ID: US 20030138907 A1 WO 200204512 A2 AU 200173348 A US 20020055145 A1 US 20020064835 A1 US 20030105017 A1

L2: Entry 15 of 15 File: DWPI Jul 24, 2003

DERWENT-ACC-NO: 2002-154921
DERWENT-WEEK: 200352
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TITLE: Purifying troponin I comprises subjecting troponin I to chromatography on anion exchanger after reversibly protecting the free sulfhydryl groups

INVENTOR: CONN, G; REARDON, B ; ZENG, X ; ZHANG, C

PRIORITY-DATA: 2000US-217069P (July 10, 2000), 2001US-0903398 (July 10, 2001), 2001US-0998619 (November 30, 2001), 2002US-0255244 (September 26, 2002), 2002US-0287118 (November 4, 2002)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
US 20030138907 A1	July 24, 2003		000	C12P021/02
WO 200204512 A2	January 17, 2002	E	028	C07K014/47
AU 200173348 A	January 21, 2002		000	C07K014/47
US 20020055145 A1	May 9, 2002		000	C12P021/02
US 20020064835 A1	May 30, 2002		000	C12P021/04
US 20030105017 A1	June 5, 2003		000	A61K038/17

INT-CL (IPC): A61 K 38/00; A61 K 38/17; C07 H 21/04; C07 K 1/16; C07 K 14/47; C12 N 1/21; C12 P 21/02; C12 P 21/04; C12 P 21/06

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments

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Term	Documents
TROPONIN	1356
TROPONINS	102
SULFHYDRYL	14036
SULFHYDRYLS	958
ISOLAT$	0
ISOLAT	37
ISOLATA	2
ISOLATABILITY	31
ISOLATABLE	2232
ISOLATABLE-TYPE	1
(TROPONIN SAME (ISOLAT$ OR PURIF$ OR CHROMOTOGRAP$) AND SULFHYDRYL).USPT,PGPB,EPAB,DWPI,TDBD.	15

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FILE 'WSCA' ENTERED AT 13:16:46 ON 24 NOV 2003
COPYRIGHT (C) 2003 PAINT RESEARCH

=> s troponin (s) (isolat? or purif? or chromotograp?) and sulfhydryl and sulfitolyz?
L1            0 FILE ADISCTI
L2            0 FILE ADISINSIGHT
L3            0 FILE ADISNEWS
L4            0 FILE AGRICOLA
L5            0 FILE ANABSTR
L6            0 FILE AQUASCI
L7            0 FILE BIOBUSINESS
L8            0 FILE BIOCOMMERCE
L9            0 FILE BIOSIS
L10           1 FILE BIOTECHDS
L11           0 FILE BIOTECHNO
L12           0 FILE CABA
L13           0 FILE CANCERLIT
L14           1 FILE CAPLUS
L15           0 FILE CEABA-VTB
L16           0 FILE CEN
L17           0 FILE CIN
L18           0 FILE CONFSCI
L19           0 FILE CROPB
L20           0 FILE CROPU
L21           0 FILE DISSABS
L22           0 FILE DGENE
L23           0 FILE DRUGB
L24           0 FILE DRUGLAUNCH
L25           0 FILE DRUGMONOG2
L26           0 FILE DRUGNL
L27           0 FILE DRUGU
L28           0 FILE DRUGUPDATES
L29           0 FILE EMBAL
L30           0 FILE EMBASE
L31           0 FILE ESBIOBASE
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'TROPONIN (S) '
L32           0 FILE FEDRIP
L33           0 FILE FOMAD
L34           0 FILE FOREGE
L35           0 FILE FROSTI
L36           0 FILE FSTA
L37           0 FILE GENBANK
L38           0 FILE HEALSAFE
L39           4 FILE IFIPAT
L40           0 FILE JICST-EPLUS
L41           0 FILE KOSMET
L42           0 FILE LIFESCI
L43           0 FILE MEDICONF
L44           0 FILE MEDLINE
L45           0 FILE NIOSHTIC
L46           0 FILE NTIS
L47           0 FILE NUTRACEUT
L48           0 FILE OCEAN
L49           0 FILE PASCAL
L50           0 FILE PCTGEN
L51           0 FILE PHAR
L52           0 FILE PHARMAML
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L53           0 FILE PHIC
L54           0 FILE PHIN
L55           0 FILE PROMT
L56           0 FILE RDISCLOSURE
L57           0 FILE SCISEARCH
L58           0 FILE SYNTHLINE
L59           0 FILE TOXCENTER
L60           4 FILE USPATFULL
L61           0 FILE USPAT2
L62           0 FILE VETB
L63           0 FILE VETU
L64           1 FILE WPIDS
L65           0 FILE 1MOBILITY
L66           0 FILE COMPENDEX
L67           0 FILE COMPUAB
L68           0 FILE CONF
L69           0 FILE ELCOM
L70           0 FILE IMSDRUGCONF
L71           0 FILE PAPERCHEM2
L72           0 FILE POLLUAB
L73           0 FILE SOLIDSTATE
L74           0 FILE ALUMINIUM
L75           0 FILE APOLLIT
L76           0 FILE AQUIRE
L77           0 FILE BABS
L78           0 FILE CAOLD
L79           0 FILE CBNB
L80           0 FILE CERAB
L81           0 FILE COPPERLIT
L82           0 FILE CORROSION
L83           0 FILE ENCOMPLIT2
L84           0 FILE INSPEC
L85           0 FILE INSPHYS
L86           0 FILE INVESTEXT
L87           0 FILE IPA
L88           0 FILE METADEX
L89           0 FILE NAPRALERT
L90           0 FILE RAPRA
L91           0 FILE RUSSCI
L92           0 FILE STANDARDS
L93           0 FILE TULSA
L94           0 FILE TULSA2
L95           0 FILE USAN
L96           0 FILE WELDASEARCH
L97           0 FILE WSCA

TOTAL FOR ALL FILES
L98          11 TROPONIN (S) (ISOLAT? OR PURIF? OR CHROMOTOGRAP?) AND SULFHYDRYL
                AND SULFITOLYZ?

=> dup rem l98
DUPLICATE IS NOT AVAILABLE IN 'ADISINSIGHT, ADISNEWS, BIOCOMMERCE, DGENE,
DRUGLAUNCH, DRUGMONOG2, DRUGUPDATES, FEDRIP, FOREGE, GENBANK, KOSMET,
MEDICONF, NUTRACEUT, PCTGEN, PHAR, PHARMAML, RDISCLOSURE, SYNTHLINE, CONF,
IMSDRUGCONF, AQUIRE, CAOLD, INVESTEXT, STANDARDS, USAN'.
ANSWERS FROM THESE FILES WILL BE CONSIDERED UNIQUE
PROCESSING COMPLETED FOR L98
L99           5 DUP REM L98 (6 DUPLICATES REMOVED)

=> d l99 1-5 ibib abs

L99  ANSWER 1 OF 5  IFIPAT  COPYRIGHT 2003 IFI on STN  DUPLICATE 1
AN                      10394487  IFIPAT;IFIUDB;IFICDB
TITLE:                  PURIFICATION OF HUMAN TROPONIN I
INVENTOR(S):            Conn; Gregory, Cary, NC, US
```

Reardon; Brian, Seattle, WA, US
Zeng; Xianfang, Northborough, MA, US
Zhang; Chenming, Blacksburg, VA, US
PATENT ASSIGNEE(S): Unassigned
AGENT: INTERVET INC, 405 STATE STREET, PO BOX 318, MILLSBORO, DE, 19966, US

	NUMBER	PK	DATE
PATENT INFORMATION:	US 2003138907	A1	20030724
APPLICATION INFORMATION:	US 2002-287118		20021104

	APPLN. NUMBER	DATE	GRANTED PATENT NO. OR STATUS
CONTINUATION OF:	US 2001-903398	20010710	
CONTINUATION OF:	US 2001-998619	20011130	

	NUMBER	DATE	
PRIORITY APPLN. INFO.:	US 2000-217069P	20000710	(Provisional)
FAMILY INFORMATION:	US 2003138907	20030724	

DOCUMENT TYPE: Utility
Patent Application - First Publication
FILE SEGMENT: CHEMICAL
APPLICATION
NUMBER OF CLAIMS: 20 11 Figure(s).
DESCRIPTION OF FIGURES:
FIGS. 1A and 1B. The chemical structure of modified cysteine. A. Conversion of cysteine to S-sulfocysteine by reaction with sodium tetrathionate and reversal by exogenous thiols. B. The cleavage of disulfide bonds by sodium sulfite to form the Ssulfo derivative.
FIG. 2. Preparation and washing of TnI-containing inclusion bodies.
FIG. 3. Summary of rTroponin-I preparation.
FIG. 4. Q-Sepharose FF chromatography Troponin I. Buffer A: 6 M urea, 25 mM Tris-HCl, pH 7.5, 100 mM; Buffer B: 6M urea, 25 mM Tris-HCl, pH 7.5, 2M NaCl; Gradient: Step; 0% B for the flowthrough and 100% B for the strip; and Flow rate: 150 ml/min.
FIG. 5. 300 ml Q-sepharose FF chromatography. Buffer A: 6M urea, 25 mM Tris-HCl, pH 7.5, 100 mM; Buffer B: 6M urea, 25 mM TrisHCl, pH 7.5, 2M NaCl; Gradient: Step; 4% B for elution and 50% B for strip; and Flow rate: 20 ml/min.
FIG. 6. SDS-PAGE analysis troponin lot after anion exchange steps no. 1 and no. 2 in 16% tris-glycine gel, under nonreducing conditions. A-H refer to lanes in the SDS-PAGE gel. A. **Sulfitolyzed** troponin Lot 3L4 standard; B. solubilized inclusion bodies; C. **sulfitolyzed** inclusion bodies (AEX No. 1 load); D. anion exchange no. 1 flowthrough; E. anion exchange no. 1 salt eulate; F. anion exchange no. 2 load; G. anion exchange no. 2 flowthrough; and, H. anion exchange no. 2 100 mM NaCl eluate.
FIG. 7. Toyopearl 650M (phenyl) HIC chromatograph. Buffer A: 6M urea, 25 mM Tris-HCl, pH 7.5, 1M (NH4)2SO4; Buffer B: 6M urea, 25 mM Tris-HCl, pH 7.5; Gradient: Step; 100% B for the flowthrough and 0% B for strip; and Flow rate: 10 ml/min.
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FIG. 9. Quantitation of rTnI on Zorbax C3.
FIG. 10. Troponin I LysC mapping.
FIG. 11. SDS-PAGE analysis of **sulfitolyzed** troponin reduction with dithiothreitol for 45 mins. at ambient temperature. One mg/ ml TnI in 6M urea, 25 mM tris, 0.15M NaCl pH 7.5, run on a 16% tris-glycine gel.

AB The invention is directed to methods for **purifying** **Troponin** I, particularly recombinant **Troponin** I

produced in a bacterial expression system. Recombinant **Troponin** I can be advantageously **purified** after reversibly protecting the free **sulfhydryl** groups, e.g., by forming sulfates. In a specific example, Tropnin I reacted with sodium tetrafhionate yields **sulfitolyzed** Tropnin I, which was **purified** by chromatography on an anion exchanger, followed by hydrophobic interaction chromatography. Facile deprotection of the **sulfhydryl** groups yields a highly **purified** product ready for refolding.

CLMN 20 11 Figure(s).

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L99 ANSWER 2 OF 5 IFIPAT COPYRIGHT 2003 IFI on STN DUPLICATE 2

AN 10360600 IFIPAT;IFIUDB;IFICDB

TITLE: **PURIFICATION** OF HUMAN **TROPONIN** I

INVENTOR(S): Conn; Gregory, Cary, NC, US
Reardon; Brian, Seattle, WA, US
Zeng; Xianfang, Northborough, MA, US
Zhang; Chenming, Blacksburg, VA, US

PATENT ASSIGNEE(S): Unassigned

AGENT: INTERVET INC, 405 STATE STREET, PO BOX 318, MILLSBORO, DE, 19966, US

	NUMBER	PK	DATE
PATENT INFORMATION:	US 2003105017	A1	20030605
APPLICATION INFORMATION:	US 2002-255244		20020926

GRANTED PATENT NO.

	APPLN. NUMBER	DATE	OR STATUS
DIVISION OF:	US 2001-903398	20010710	
FAMILY INFORMATION:	US 2003105017	20030605	
DOCUMENT TYPE:	Utility Patent Application - First Publication		
FILE SEGMENT:	CHEMICAL APPLICATION		
NUMBER OF CLAIMS:	20 11 Figure(s).		

DESCRIPTION OF FIGURES:

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CLMN 20 11 Figure(s).

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FIG. 5. 300 ml Q-sepharose FF chromatography. Buffer A: 6M urea, 25 mM Tris-HCl, pH 7.5, 100 mM; Buffer B: 6M urea, 25 mM TrisHCl, pH 7.5, 2M NaCl; Gradient: Step, 4% B for elution and 50% B for strip; and Flow rate: 20 ml/min.

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L99 ANSWER 3 OF 5 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2002-08599 BIOTECHDS
TITLE: **Purifying troponin** I comprises subjecting **troponin** I to chromatography on anion exchanger after reversibly protecting the free **sulfhydryl** groups; recombinant production in Escherichia coli and application in e.g. cancer therapy
AUTHOR: CONN G; REARDON B; ZENG X; ZHANG C
PATENT ASSIGNEE: DIOSYNTH RTP INC
PATENT INFO: WO 2002004512 17 Jan 2002
APPLICATION INFO: WO 2000-US21817 10 Jul 2000
PRIORITY INFO: US 2000-217069 10 Jul 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-154921 [20]
AN 2002-08599 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - Preparing **troponin** I, comprising protecting free **sulfhydryl** groups of **troponin** I under reducing conditions, and **troponin** I is then **purified** by subjecting **troponin** I comprising **sulfhydryl** protecting groups to chromatography, is new.

DETAILED DESCRIPTION - An INDEPENDENT CLAIM is also included for **troponin** I comprising **sulfhydryl** protecting groups.

BIOTECHNOLOGY - Preferred Method: The recombinant **troponin** I is expressed in a bacterial expression system, preferably an Escherichia coli expression system. The free **sulfhydryl** groups are protected by **sulfitolyzation** which comprises reacting reduced recombinant **troponin** I with sodium tetrathionate. **Troponin** I is **purified** by chromatography under non-reducing conditions and the **sulfhydryl** groups are deprotected from the **purified troponin** I. The chromatographic support is an anion exchange column, optionally followed by hydrophobic interaction chromatography. **Troponin** I is

denatured and the **sulfhydryl** protecting groups are sulfates.

ACTIVITY - Cytostatic.

MECHANISM OF ACTION - Inhibitor of angiogenesis. No supporting data is given.

USE - The method is useful for **purifying troponin** I, particularly recombinant **troponin** I. The highly **purified troponin** I, preferably in a refolded state is useful for antibody generation, as a control or standard immunoassay reagent, or to inhibit angiogenesis important in treating various cancers.

ADVANTAGE - Protection of **sulfhydryl** groups during **troponin** I preparation eliminates the costly need for maintaining non-reducing conditions throughout protein preparation, **purification** and storage, and need for reducing agents. The **sulfhydryl**-protected **troponin** does not form intrachain or interchain disulfide crosslinks. Overall yield of **troponin** from the multi-step **purification** was greater than 50% at purity levels of greater than 95%. Deprotection of the **sulfhydryl** groups yields a highly **purified** product ready for refolding.

EXAMPLE - Human skeletal **troponin** I (TnI) expressed in Escherichia coli was **isolated** from lysed cells in inclusion bodies. 10 g of TnI-containing inclusion bodies were solubilized and protein **sulfhydryls** were **sulfitolyzed** using 6 M urea (200 ml), Tris (25 mM), sodium sulfite (10 mg/ml), sodium tetrathionate (5 mg/ml) pH 7.5 at ambient temperature for 6 hours in the dark. The solubilized material was filtered over a 0.2 micro membrane prior to subsequent processing. **Sulfitolyzed** recombinant human TnI was **purified** by a five step process. Solubilized, **sulfitolyzed** TnI-containing inclusion bodies (200 ml) were loaded onto a 3 l volume Q-sepharose FF column pre-equilibrated in 6 M urea, 25 mM Tris, 0.1 M NaCl pH 7.5 at 150 ml/min. The **purified** TnI was collected in the column flowthrough. The recovered TnI was concentrated. This material was loaded onto a 300 ml volume Q-sepharose FF column pre-equilibrated in 6M urea, 25 mM Tris, pH 7.5 at 20 ml/minute. The bound TnI was eluted from the column by a step wash with 6 M urea, 25 mM Tris, 80 mM NaCl pH 7.5. This eluted **troponin** (500 ml) was loaded onto a 60 ml column of Toyopearl 650 M phenyl HIC resin after addition of ammonium sulfate to a final concentration of 1 M. The column was pre-equilibrated with 6 M urea, 25 mM Tris, 1M ammonium sulfate pH 7.5. The **purified troponin** was collected as the unbound flowthrough from this column, concentrated 2.5-fold and buffer exchanged for storage by UF/DF. **Purified** TnI was stored frozen at -70 degrees C. Protein purity was determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and reverse phase chromatography and protein identity was confirmed by peptide mapping with peptide mass and fragmentation analysis. Yield determinations for each step were determined by quantitative reverse phase chromatography. Residual DNA levels, measured by DNA threshold, were less than or equal to 12 pg DNA/mg protein. Endotoxin testing of final product by Limulus Amoebocyte Lysate (LAL) (gel-clot) indicated less than or equal to 3 EU/mg protein. (28 pages)

L99 ANSWER 4 OF 5 IFIPAT COPYRIGHT 2003 IFI on STN DUPLICATE 4

AN 10121228 IFIPAT;IFIUDB;IFICDB

TITLE: **PURIFICATION** OF HUMAN **TROPONIN** I; GENERATING MUSCLE PROTEIN; OBTAIN SAMPLE, INCUBATE UNDER REDUCING ENVIRONMENT, RECOVER MUSCLE PROTEIN

INVENTOR(S): Conn; Gregory, Cary, NC, US
Reardon; Brian, Seattle, WA, US
Zeng; Xianfang, Northborough, MA, US
Zhang; Chenming, Blacksburg, VA, US

PATENT ASSIGNEE(S): Diosynth RTP, Inc.

AGENT: DARBY & DARBY P.C., 805 Third Avenue, New York, NY, 10022, US

	NUMBER	PK	DATE
PATENT INFORMATION:	US 2002064835	A1	20020530
APPLICATION INFORMATION:	US 2001-903398		20010710

	NUMBER	DATE
PRIORITY APPLN. INFO.:	US 2000-217069P	20000710 (Provisional)
FAMILY INFORMATION:	US 2002064835	20020530

DOCUMENT TYPE: Utility
Patent Application - First Publication
FILE SEGMENT: CHEMICAL
APPLICATION
NUMBER OF CLAIMS: 20 11 Figure(s).
DESCRIPTION OF FIGURES:
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CLMN 20 11 Figure(s).
FIGS. 1A and 1B. A. Proposed reaction for oxidative sulfitolysis. B. Cleavage of disulfide bond by sodium sulfite to form the Ssulfo derivative.
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FIG. 3. Summary of rTroponin-I preparation.
FIG. 4. Q-Sepharose FF chromatography of Troponin I. Buffer A: 6 M urea, 25 mM Tris-HCl, pH 7.5, 100 mM; Buffer B: 6 M urea, 25 mM Tris-HCl, pH 7.5, 2 M NaCl; Gradient: Step, 0% B for the flow-through and 100% B for the strip; and Flow rate: 150 ml/ min.
FIG. 5. 300 ml Q-sepharose FF chromatography. Buffer A: 6 M urea, 25 mM Tris-HCl, pH 7.5, 100 nM; Buffer B: 6 M urea, 25 mM TrisHCl, pH 7.5, 2 M NaCl; Gradient: Step, 4% B for elution and 50% B for strip; and Flow rate: 20 ml/min.
FIG. 6. SDS-PAGE analysis troponin lot after anion exchange steps no. 1 and no. 2 in 16% tris-glycine gel, under nonreducing conditions. A-H refer to lanes in the SDS-PAGE gel. A. **Sulfitolyzed** troponin Lot 3L4 standard; B. solubilized inclusion bodies; C. **sulfitolyzed** inclusion bodies (AEX No. 1 load); D. anion exchange no. 1 flowthrough; E. anion exchange no. 1 salt eulate; F. anion exchange no. 2 load; G. anion exchange no. 2 flowthrough; and, H. anion exchange no. 2 100 mM NaCl eluate.
FIG. 7. Toyopearl 650 M (phenyl) HIC chromatograph. Buffer A: 6 M urea, 25 mM Tris-HCl, pH 7.5, 1 M (NH4)2SO4; Buffer B: 6 M urea, 25 mM Tris-HCl, pH 7.5; Gradient: Step, 100% B for the flow-through and 0% B for strip; and Flow rate: 10 ml/min.
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FIG. 9. Quantitation of rTnI on Zorbax C3.
FIG. 10. Troponin I LysC mapping.
FIG. 11. SD S-PAGE analysis of **sulfitolyzed** troponin reduction with dithiothreitol for 45 mins. at ambient temperature. One mg/ ml TnI in 6 M urea, 25 mM tris, 0.15 M NaCl pH 7.5, run on 16% tris-glycine gel. 1. 10., Mark 12 MW Stds; 2. 9., **sulfitolyzed** TnI; 3. 0.05 mM DTT; 4. 0.10 mM DTT; 5. 0.2 mM DTT; 6. 0.3 mM DTT; 7. 0.5 mMDTT; 8. 1.0 mM DTT.

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TITLE:	**PURIFICATION** OF HUMAN **TROPONIN** I; **ISOLATING** PREFERENTIAL POLYPEPTIDE; OBTAIN SAMPLE, INCUBATE WITH ION EXCHANGE RESIN, ELUTE, RECOVER POLYPEPTIDE
INVENTOR(S):	Conn; Gregory, Cary, NC, US Reardon; Brian, Seattle, WA, US Zeng; Xianfang, Northborough, MA, US Zhang; Chenming, Blacksburg, VA, US
PATENT ASSIGNEE(S):	Diosynth RTP, Inc.
AGENT:	DARBY & DARBY P.C., 805 Third Avenue, New York, NY, 10022, US

	NUMBER	PK	DATE
PATENT INFORMATION:	US 2002055145	A1	20020509
APPLICATION INFORMATION:	US 2001-998619		20011130

	APPLN. NUMBER	DATE	GRANTED PATENT NO. OR STATUS
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PRIORITY APPLN. INFO.:	US 2000-217069P	20000710	(Provisional)
FAMILY INFORMATION:	US 2002055145	20020509	
DOCUMENT TYPE:	Utility		

Patent Application - First Publication
FILE SEGMENT: CHEMICAL
APPLICATION
NUMBER OF CLAIMS: 20 11 Figure(s).
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